Attorney's Docket No.: 10276-057001

PROVISIONAL APPLICATION FOR PATENT

under

37 CFR §1.53(c)

TITLE: MODULATORS OF P85 EXPRESSION

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MODULATORS OF P85 EXPRESSION

Field of the Invention

The invention relates to methods of diagnosing and treating insulin-related disorders.

Background of the Invention

The treatment of insulin resistant states and type 2 diabetes remains problematic. Basic phatophysiologic studies have suggested that a main component, perhaps the earliest component, in the development of type 2 diabetes is insulin resistance. Among currently available agents for the treatment of type 2 diabetes, thiazolidiones are directed to improving insulin sensitivity. This class of agents works through the mechanism of increasing the expression of some insulin sensitive genes, in particular, glucose transporter genes. The biguadides, such as Metformin, also have some effects on insulin-sensitive tissues, especially the liver, but their mechanism of action remains unknown. The treatment of patients having type 2 diabetes frequently requires multiple agents, and even with these agents, the control of blood glucose is often poor. In addition to type 2 diabetes, insulin resistance is common to a number of other conditions, such as obesity, hypertension, polycystic ovarian disease, and various hypolipidemias.

Summary

In general, the invention features a method of treating a subject having an insulin-related disorder. An insulin-related disorder as defined herein includes diabetes, e.g., type 2 diabetes, and atypical insulin resistant states. The method includes: altering the ratio of p85α with respect to one or more of p110, p85β, or IRS. As used herein, "altering" can mean increasing or decreasing the amount of p85α, e.g., increasing or decreasing the level of p85α mRNA and/or p85α protein expression, but preferably means decreasing. A reduction in the availability of p85α results in improved insulin sensitivity and glucose uptake.

In a preferred embodiment, the method includes: decreasing the amount of active p85α in a cell of a subject, e.g., by administering a compound which binds and thereby

inhibits or sequesters the p85α. "Active p85α" refers to p85α in a cell available for interacting with p110 as part of the PI3K signaling cascade. The amount of active p85α can be decreased by either decreasing the total amount of p85α in a cell and/or by inhibiting the functional activity of p85α that is present in a cell.

Compounds which bind, and preferably thereby inhibit or sequester, p85α can be used to decrease p85α. Such compounds can include: anti-p85α antibodies, soluble fragments of p85α ligands, e.g., p110, small molecules, and random peptides selected, e.g., selected in a phage library, for the ability to bind to p85α.

Peptides are examples of compounds which can bind, inhibit and/or sequester p85α. For example, peptide fragments of p110 or small peptides that have been selected on the basis of binding p85α can be used. These can be selected in phage display or by similar methods. Such peptides are preferably at least four, more preferably at least six or ten amino acid residues in length. They are preferably less than 100, more preferably less than 50 and most preferably less than 30 amino acids in length. Preferably, the peptide inhibits the ability of p85α to interact with, e.g., bind to, a p85α ligand, e.g., p110. In one embodiment, the peptide binds to an active domain of p85α, e.g., an SH2 domain, an SH3 domain, a Rho-GAP homology domain, and/or a polyproline domain.

Small molecules can also be used. "Small molecules", as used herein, refers to a non-peptide compound which is preferably of less than 5,000, more preferably less than 2,500, most preferably less than 1,500 in molecular weight. Preferably, a small molecule binds to p85α and inhibits at least one of its wild-type functions, e.g., inhibits an interaction with p110. Preferably, the interaction between the small molecule and p85α results in increased insulin sensitivity. In one embodiment, the small molecule binds to an active domain of p85α, e.g., an SH2 domain, an SH3 domain, a Rho-GAP homology domain, and/or a polyproline domain.

The level of free or active p85α can also be reduced by administration of a nucleotide sequence which binds to and inhibits p85α expression, e.g., a p85α antisense molecule. In preferred embodiments, the p85α antisense molecule is delivered by, e.g., gene or cell therapy. In other embodiments, the p85α antisense molecules are delivered by the administration of the oligonucleotides.

The level of P85α expression can also be inhibited by decreasing the level of expression of an endogenous P85α gene, e.g., by decreasing transcription of the P85α gene. In a preferred embodiment, transcription of the P85α gene can be decreased by: altering the regulatory sequences of the endogenous P85α gene, e.g., by the addition of a negative regulatory sequence (such as a DNA-binding site for a transcriptional repressor).

In another preferred embodiment, the invention further includes: increasing the level of p85β in a cell of the subject. The level of p85β can be increased by, e.g., providing a nucleic acid encoding p85β or a functional fragment or analog thereof and/or a p85β protein or functional fragment or analog thereof. A nucleic acid encoding p85β or a functional fragment or analog thereof can be delivered, e.g., by gene or cell therapy. Alternatively, the level of p85β can be increased by providing a substance that increases transcription of p85β. In a preferred embodiment, transcription of p85β is increased by: altering the regulatory sequences of the endogenous p85β gene, e.g., by the addition of a positive regulatory element (such as an enhancer or a DNA-binding site for a transcriptional activator); the deletion of a negative regulatory element (such as a DNA-binding site for a transcriptional repressor) and/or replacement of the endogenous regulatory sequence, or elements therein, with that of another gene, thereby allowing the coding region of the p85β gene to be transcribed more efficiently. In another preferred embodiment, the level of p85β can be increased by, e.g., providing an agent which increases the level of p85β, e.g., a small molecule which binds to the promoter region of p85β.

In a preferred embodiment, the method further includes: increasing the level of p110 in a cell of the subject. Levels of p110 can be increased by, e.g., providing a nucleic acid encoding p110 or a functional fragment or analog thereof and/or a p110 protein or functional fragment or analog thereof. A nucleic acid encoding p110, or a functional fragment or analog thereof, can be delivered by, e.g., gene or cell therapy. Alternatively, the level of p110 can be increased by providing a substance that increases transcription of p110. In a preferred embodiment, transcription of p110 is increased by: altering the regulatory sequences of the endogenous p110 gene, e.g., by the addition of a positive regulatory element (such as an enhancer or a DNA-binding site for a transcriptional activator); the deletion of a negative regulatory element (such as a DNA-

binding site for a transcriptional repressor) and/or replacement of the endogenous regulatory sequence, or elements therein, with that of another gene, thereby allowing the coding region of the p110 gene to be transcribed more efficiently. In another preferred embodiment, the level of p110 can be increased by, e.g., providing an agent which increases the level of p110, e.g., a small molecule which binds to the promoter region of p110.

In preferred embodiments, the subject has exhibited at least one indication of an insulin-related disorder, e.g., insulin resistance, prior to receiving a treatment provided herein. In one embodiment, the subject has type 2 diabetes.

In other embodiments, a treatment described herein is provided to a subject in the absence of the subject having exhibited symptoms of an insulin-related disorder. In one embodiment, the subject is thought to be at risk for an insulin-related disorder, e.g., insulin resistance.

In another aspect, the invention features a method of treating a subject having an insulin-related disorder. The method includes: increasing the level of p85β in a cell of the subject.

In a preferred embodiment, the level of p85β is increased by providing a nucleic acid encoding p85β or a functional fragment or analog thereof, or by providing a substance that induces transcription of p85β. In a preferred embodiment, transcription of p85β is increased by: altering the regulatory sequences of the endogenous p85β gene, e.g., by the addition of a positive regulatory element (such as an enhancer or a DNA-binding site for a transcriptional activator); the deletion of a negative regulatory element (such as a DNA-binding site for a transcriptional repressor) and/or replacement of the endogenous regulatory sequence, or elements therein, with that of another gene, thereby allowing the coding region of the p85β gene to be transcribed more efficiently. In another preferred embodiment, the level of p85β can be increased by, e.g., providing an agent which increases the level of p85β, e.g., a small molecule which binds to the promoter region of p85β. The level of p85β can also be increased by, e.g., providing a p85β protein or functional fragment or analog thereof.

In preferred embodiments, the nucleic acid encoding p85β or a functional fragment or analog thereof is delivered by, e.g., gene or cell therapy.

In a preferred embodiment, the method includes decreasing the amount of active p85α in the subject. The amount of active p85α can be decreased by, for example, any of the methods described herein.

In another embodiment, the method includes increasing the amount of p110 in a cell of the subject. In a preferred embodiment, the level of p110 is increased by providing a nucleic acid encoding p110 or a functional fragment or analog thereof, or by providing a substance that induces transcription of p110. In a preferred embodiment, transcription of p110 is increased by: altering the regulatory sequences of the endogenous p110 gene, e.g., by the addition of a positive regulatory element (such as an enhancer or a DNA-binding site for a transcriptional activator); the deletion of a negative regulatory element (such as a DNA-binding site for a transcriptional repressor) and/or replacement of the endogenous regulatory sequence, or elements therein, with that of another gene, thereby allowing the coding region of the p110 gene to be transcribed more efficiently. In another preferred embodiment, the level of p110 can be increased by, e.g., providing an agent which increases the level of p110, e.g., a small molecule which binds to the promoter region of p110. The level of p110 can also be increased by, e.g., providing a p110 protein or functional fragment or analog thereof.

In preferred embodiments, the nucleic acid encoding p110 or a functional fragment or analog thereof is delivered by, e.g., gene or cell therapy.

In another aspect, the invention provides a method of determining if a subject is at risk for a disorder, e.g., an insulin-related disorder, e.g., a disorder related to a lesion in or the misexpression of the gene which encodes p85α.

Such disorders include, e.g., a disorder associated with the misexpression of p85α; a disorder associated with glucose uptake; and/or a disorder associated with insulin sensitivity such as type 2 diabetes.

In a preferred embodiment, the method includes evaluating the expression of p85α to determine if the subject is at risk, to thereby determine if a subject is at risk.

In a preferred embodiment, the method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the p85α gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure or expression of the p85α gene;

detecting, in a tissue of the subject, the misexpression of the p85α gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA, e.g., wherein increased levels of p85α mRNA is associated with decreased insulin sensitivity, e.g., is indicative of a risk of type 2 diabetes;

detecting, in a tissue of the subject, the misexpression of the p85α gene, at the protein level, e.g., detecting a non-wild type level of a p85α polypeptide, wherein increased levels of p85α protein is associated with decreased insulin sensitivity, e.g., is indicative of a risk of type 2 diabetes.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the p85α gene; an insertion of one or more nucleotides into the gene; a point mutation, e.g., a substitution of one or more nucleotides of the gene; a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer, e.g., a labeled probe/primer, which includes a region of nucleotide sequence which hybridizes to a sense or antisense sequence from the p85α gene, or naturally occurring mutants thereof, or to the 5' or 3' flanking sequences naturally associated with the p85α gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In a preferred embodiment, detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the p85α gene, e.g., as compared to levels in a subject not at risk for an insulin related disorder; the presence of a non-wild type splicing pattern of a messenger RNA transcript

of the gene; or a non-wild type level of the p85α protein e.g., as compared to levels in a subject not at risk for an insulin related disorder.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In a preferred embodiment, the method includes determining the structure of a p85α gene, an abnormal structure being indicative of risk for the disorder.

In a preferred embodiment, the method includes contacting a sample from the subject with an antibody to the p85α protein or a nucleic acid, which hybridizes specifically with a portion of the gene.

In another aspect, the invention features a method of analyzing a treatment for its effect, e.g., for its effect on insulin metabolism, e.g., insulin sensitivity or glucose uptake, in a subject. The method includes providing an animal or a cell, in which the ratio of p85α to one or more of p110, p85β, or IRS has been altered. Preferably, the ratio of p85α to any of p110, p85β, and IRS has been decreased. In a preferred embodiment, the subject is a genetically modified animal having a genetic lesion, for example a knockout, at the gene which encodes p85α. This animal may be useful to compare the effectiveness of a treatment in a wild type animal, wherein the treatment is designed to reduce the amount of active p85α.

A treatment, e.g., a compound administered to the subject, can be evaluated for its effect on insulin metabolism, for example, insulin sensitivity.

In another embodiment, the subject is a transgenic animal, e.g., a transgenic rodent, e.g., mouse, having a transgene, for example a transgene which encodes p85β.

In another embodiment, the subject is a transgenic animal, e.g., a transgenic rodent, e.g., mouse, having a transgene, for example, a transgene which encodes p85α. In this embodiment, the transgenic mouse may be useful as a model for decreased insulin sensitivity, e.g., type 2 diabetes.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Detailed Description

The invention provides methods of modulating the expression of class 1_API3K regulatory subunit genes or inhibiting the function of various domains of class 1_API3K regulatory subunit molecules as a treatment for insulin resistance and type 2 diabetes.

Phosphoinositide 3-kinases (PI3Ks) are enzymes that phosphorylate the D-3 position of phospholipids containing an inositol headgroup (phosphoinositides). PI3Ks are involved in many cellular responses triggered by external stimuli. For example, insulin-dependent glucose uptake is thought to require PI3K activation. Several classes of PI3Ks exist in mammalian cells. Class I_API3Ks are heterodimers of a catalytic subunit of about 110 kDa (p110) and a regulatory subunit, usually of about 85 kDa (p85).

The role of PI3K in insulin signaling is as follows. The insulin receptor tyrosine kinase is activated by binding of insulin to the extracellular region of it receptor. The activated tyrosine kinase phosphorylates insulin receptor substrate (IRS) proteins on numerous phosphotyrosine (pTyr) residues. Some of these are specific binding sites for the Src-homology-2 (SH2) domains of class I_A regulatory subunits. Association of PI3K with IRS proteins increases the lipid kinase activity of the p110 subunit and brings it into proximity with substrates at the membrane. The lipid products act as second messengers to recruit other signaling proteins to the membrane. This signaling eventually leads to glucose uptake by the cell. The importance of PI3K in this signaling process is supported by two general types of experiments. First, compounds that inhibit p110 kinase activity (e.g., wortmannin, Ly294002) block insulin-mediated glucose transport in cultured cells. Second, expression of constitutively active forms of PI3K can stimulate glucose transport and dominant negative forms can inhibit glucose transport.

Pik3rl Knockout Mice

Deletion of class I_A regulatory subunits by gene targeting was predicted to result in insulin resistance and possibly diabetes, as is seen in mice lacking the insulin receptor or certain IRS proteins. To test this, mice were created which lacked the *Pik3r1* gene. The *Pik3r1* gene encodes the isoforms p85α, p55α, and p50α. Surprisingly, the mice were hypoglycemic, despite lower serum insulin levels in the fed state. Fasted animals show enhanced glucose disposal in a glucose tolerance test, while maintaining lower insulin levels. Insulin sensitivity could not be tested directly in *Pik3r1* -/- mice because homozygous mice died before adulthood. Biochemical studies of insulin-stimulated liver and muscle revealed normal activation of the PI3K downstream target Akt/PKB in *Pik3r1* -/- mice, suggesting that the output of PI3K signaling is unimpaired *in vivo*, despite disruption of the *Pik3r1* gene. The expression of the genes encoding p85β and p55γ regulatory isoforms was increased in the liver and muscle of *Pik3r1* -/- mice, thus providing a possible compensatory mechanism.

Pik3r1 +/- mice were viable, exhibiting reduced expression of *Pik3r1* gene products and had some increase in p85β expression. These mice demonstrated hypoglycemia, although the hypoglycemia was milder than that detected in the *Pik3r1* -/- mice. The *Pik3r1* +/- mice exhibited improved glucose tolerance relative to their wild-type littermates. Insulin tolerance tests showed a significant increase in insulin sensitivity in *Pik3r1* +/- mice.

The presence of a single disrupted allele of *Pik3r1* (*Pik3r1* +/-) improved insulin sensitivity in three separate models of insulin resistance in mice: (1) Insulin receptor heterozygotes (IR +/-), insulin receptor substrate-1 heterozygotes (IRS-1 +/-), and IR/IRS-1 double heterozygotes. In the IR/IRS-1 double heterozygotes, overt diabetes was prevented in ~50% of the IR/IRS-1/*Pik3r1* heterozygotes.

The studies of the heterozygous and homozygous mice described above suggest that altering the balance of expression of PI3K regulatory isoforms can influence the sensitivity of insulin signaling *in vivo*. Several molecular mechanisms could explain these results. For instance, it is possible that class I_A regulatory proteins are normally in excess in insulin-responsive tissues. If this were the case, the free subunits would

compete for binding to phosphorylated IRS proteins with heterodimeric class I_A complexes and possibly with other SH2 domain-containing signaling proteins. Reducing the abundance of the free regulatory subunits would allow more efficient activation of PI3K and possibly other targets. It is also possible that upregulation of isoforms that are normally of low abundance in insulin-responsive tissues, such as p85β and p55γ, affects the functional response because of altered recruitment of modifying signaling molecules and/or subcellular localization. Regardless of the mechanism of increased insulin sensitivity, drugs that affect expression or function of individual PI3K isoforms are useful in the treatment of insulin-resistance syndromes such as type 2 diabetes

The knockout mice described herein can be used in various ways. For example, the mice can be used as a benchmark with which to compare drugs that regulate PI3K subunit expression. For example, a drug that causes reduced p85α expression or increased p85β expression might effect an increase insulin sensitivity in diabetic animals.

The knockout mice can also be used to develop drugs that modulate function of subdomains of PI3K regulatory isoforms, such as SH2, SH3, Rho-GAP homology and polyproline domains.

A number of methods could be employed to alter the expression of p85α or the functional interaction between our p85α and p110α. These methods include, for example the use of antisense or ribozymes. Other approaches include, e.g., the use of small molecules which regulate gene expression at the post-transcriptional level.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.